

Natural killer cells in meningiomas

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Summary. A monoclonal antibody against the surface marker IOT-10 of natural killer (NK) cells was used to investigate the presence of these cells in a series of twenty intracranial meningiomas. In all of these tumours, IOT-10 positive NK cells were found in small numbers, mainly distributed among the tumor cells. Two recurrent tumors showed a relatively high number of immunostained cells. The data obtained in the present study suggest that a NK-cell-mediated immunological response can occur in meningioma tissue.

Key words: Natural Killer Cell, Monoclonal antibody, Lymphocyte, Brain tumors, Meningioma

Introduction

Information about phenotypic characterization of lymphocytes infiltrating brain tumors has been reported in the last years (Stavrou et al., 1977; von Hanwehr et al., 1984; Paine et al., 1986; Ullén et al., 1986; Bhondeley et al., 1988; Hitchcock and Morris, 1988). Nevertheless, few studies have been reported on the presence of natural-killer (NK) cells in intracranial tumors, despite the fact that these cells are a subpopulation of lymphocytes with spontaneous cytotoxic capacity against the tumoral cell, suggesting a particular role in the regulation of proliferation and tumor surveillance (Hoffmann and Ferrarini, 1983; Lotzová, 1983).

Using a monoclonal antibody against the surface marker IOT-10 of NK cells, we have previously studied the presence of these cells in glioblastomas (Vaquero et al., 1989), intracranial germinomas (Vaquero et al., 1990b), and brain metastases (Vaquero et al., 1990a), suggesting that NK-cells did not play a significant role in the cellular infiltrate or biological prognosis of these

neoplasms. The immunohistochemical demonstration of the presence of NK-cells in intracranial meningiomas is the aim of the present report.

Materials and methods

Twenty tumor samples from 17 patients with intracranial meningioma were studied. All the patients underwent a radical resection of convexity or parasagittal meningioma, and all the patients received steroids (dexamethasone, 16 mg/day, IM) in the days prior to surgery.

In the present series, the female/male ratio was 12/5, and age, when tumor was resected, ranged between 36 and 64 years (mean: 54 years).

Fifteen patients were operated on for a single tumor, that showed no recurrence after a follow-up of more than five years. Case 16 of the series was a 38-year-old man, operated on for a convexity frontoparietal transitional meningioma, with angiomatous areas, that showed a tumoral recurrence one year later with histological features similar to the previous one (Fig. 1). Two years after the last operation, he is symptom-free. In this patient, both resected tumors were included in the series. Case 17 of the series corresponded to a female patient, diagnosed with multiple meningiomas, that underwent craniotomies for resection of transitional meningiomas in the right parasagittal region, tentorium, and right parasagittal region again, at ages of 56, 57, and 58 years, respectively (Fig. 2). The three resected meningiomas of this patient were also included in the present study.

From each tumor, two paraffin-embedded slices were processed for phenotypic identification of the surface marker IOT-10 of NK-cells.

The avidin-biotin-peroxidase complex (ABC) technique was employed. Specific murine monoclonal antibody (Immunotech, Marseille) diluted 1:50 was used. After being dewaxed the sections were hydrated with graded alcohol series and rinsed for 5 minutes in distilled water. After this, sections were incubated for

Table 1. Histological subtypes and degree of IOT-10-positive-NK-cell infiltration in the meningiomas of the series. The degree of NK-cell infiltration is expressed by the number of immunostained cells for every 10 microscopic fields, at $\times 400$ (+): 1 to 5 immunostained cells. (++) : 5 to 10 immunostained cells. (+++) : more than 10 immunostained cells.

Case	Histological subtype	Degree of NK-Cell infiltration
1	psammomatous	(+)
2	meningotheliomatous	(+)
3	meningotheliomatous	(+)
4	fibrous	(+)
5	fibrous	(+)
6	fibrous	(++)
7	transitional	(+)
8	transitional	(+)
9	transitional	(+)
10	transitional	(+)
11	transitional	(+)
12	transitional	(+)
13	transitional	(+)
14	transitional	(+)
15	transitional	(+)
16	transitional with angiomatous areas	(+)
	transitional with angiomatous areas	(+++)
17	transitional	(++)
	transitional	(++)
	transitional	(+++)

30 minutes in 0.3% H_2O_2 in methanol, and washed in phosphate-buffered saline (PBS) for 20 minutes. Normal horse serum (3%) was applied for 20 minutes to decrease nonspecific background staining, followed by incubation with primary antibody for 30 minutes in a moist chamber. This was followed by washing in PBS for 15 minutes, repeated three times. Secondary biotinylated horse antibody against mouse immunoglobulins (Vector Lab., Burlingame, CA) was then applied for 20 minutes, followed by a 45-minute PBS wash. Avidin-peroxidase complex (ABC reagent, Vectastain, Vector Lab., Burlingame, CA) was added for 20 minutes followed by washing for 60 minutes in four 15 minute intervals. 3-amino-9-ethylcarbazole (AEC) was added for 5 minutes, followed by a 5-minute wash, and finally hematoxylin was added for 2 minutes. The slices were mounted with aqueous mounting medium, and observed under the microscope. Positive controls consisted of human tonsil, and negative controls were obtained by incubating the tumor slices with normal mouse serum diluted 1:50 with PBS instead of the primary antibody.

A search for IOT-10-positive NK cells was

conducted in each tumor by two different observers. The presence or absence of these cells was described as: 0 (absence of immunostained cells), + (from 1 to 5 immunostained cells for every 10 microscopic fields, at $\times 400$), ++ (from 5 to 10 immunostained cells), and +++ (more than 10 immunostained cells). A minimum of 100 microscopic fields were studied from each tumor.

In each case, the histological features of the tumor and the degree of lymphocytic infiltration, in H & E stained slices, was recorded.

Results

In the present series, all the tumoral samples studied showed IOT-10-positive NK-cells, but these cells were generally found in small numbers and scattered among the tumor cells (Table I) (Fig. 3).

Only two tumoral samples showed a significant degree of immunostained cell infiltration (mean of 1.8 and 1.4 immunostained cells per microscopic field, at $\times 400$). They corresponded to tumor recurrences of patients 16 and 17, respectively, whose clinical data have been summarized above.

When lymphocytic infiltration was analyzed in H & E stained slices, only one tumor showed it to be significant. This tumor corresponded to the recurrence of case 16. In this one immunostained cells were associated with the tissular presence of lymphocytes, but most IOT-10-positive NK-cells were localized among the tumor cells, in zones without mononuclear cell infiltration.

On the other hand, when the degree of immunostained cells was correlated with clinical data such as sex or age of the patients, or with histological data, such as the different subtypes of meningiomas, no evidence of correlation was found.

Discussion

The first conclusion we obtain from this study is that the presence of IOT-10-positive NK-cells is a common finding in meningiomas, and that most of them are dispersed among the tumoral cells. Although it must be remembered that each tumor was only studied partially, the incidence of NK-cells in the present series is higher than that reported by Stevens et al. (1988), in the sole previous study reporting the presence of NK-cells in meningiomas; they found that only 45% of the meningiomas studied had NK-cells.

In our series, the pattern of tissular distribution of NK-cells, showing immunostained cells masquerading among the tumor cells, is similar to that previously found by us in glioblastoma (Vaquero et al., 1989), intracranial germinoma (Vaquero et al., 1989b), and brain metastases (Vaquero et al., 1990a). Nevertheless, in glioblastoma, intracranial germinoma and brain metastases, the degree of NK-cell infiltration clearly does not correlate with the degree of lymphocytic infiltration in the H & E stained slices

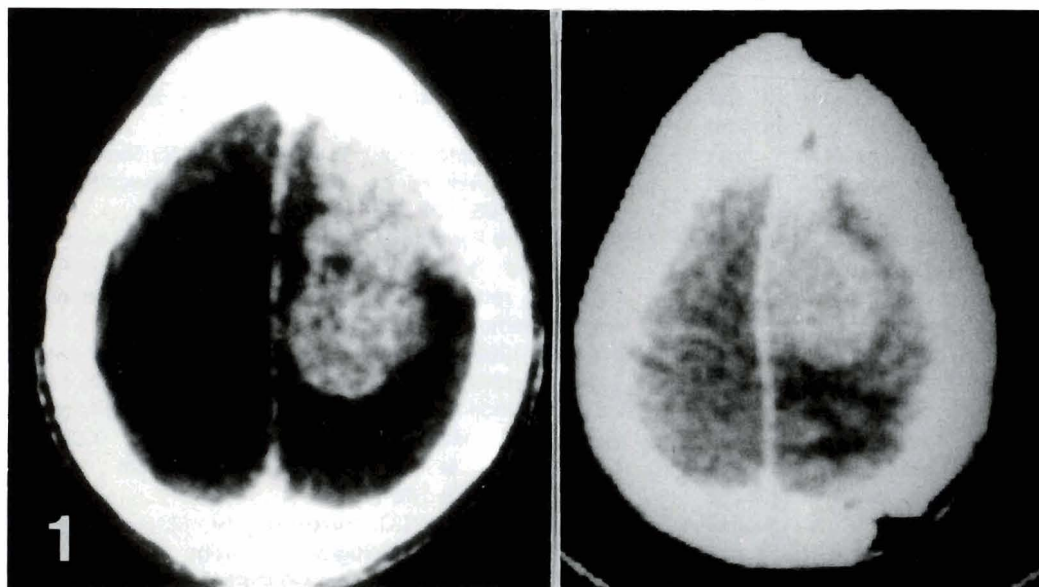


Fig. 1. Case 16. Left: CT-scan, after contrast administration, showing a right convexity frontoparietal meningioma. Right: CT-scan, after contrast administration, two years later, showing tumor recurrence. The degree of IOT-10-positive NK-cells was estimated as (+) in the original tumor, and (+++) in the recurrent tumor.

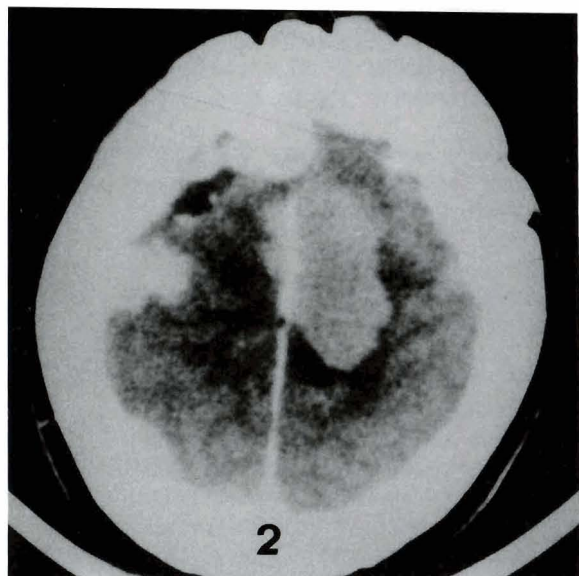


Fig. 2. Case 17. CT-scan, after contrast administration, showing multiple meningiomas. Right parasagittal mass is a recurrent meningioma showing a degree (+++) of IOT-10-positive NK-cell infiltration.

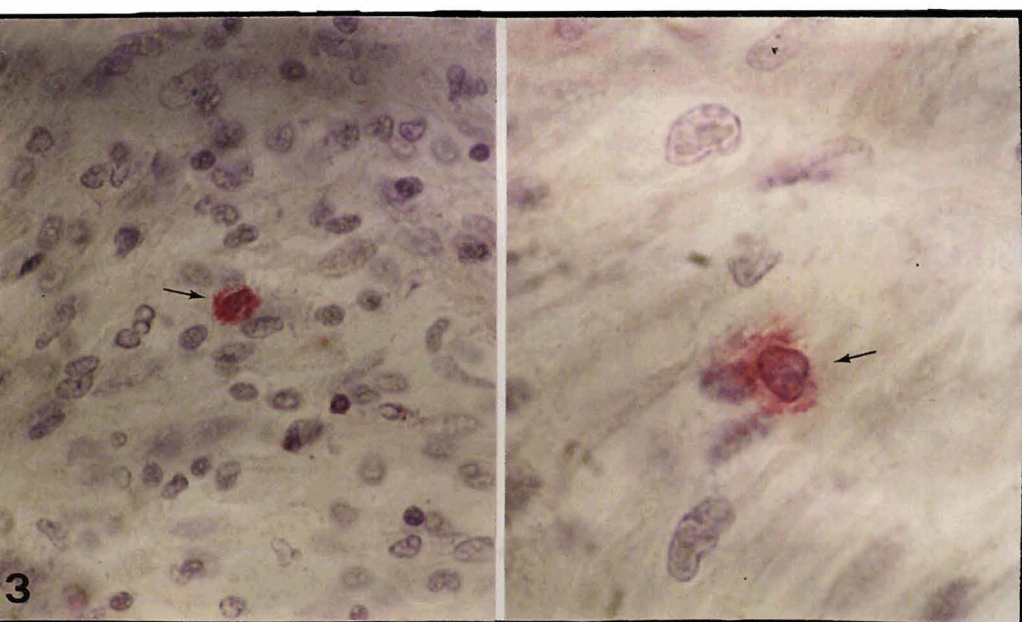


Fig. 3. Immunohistochemical demonstration of IOT-10-positive NK-cells in meningiomas. The immunostained cells (arrows) were scattered among the tumor cells. ABC technique, original magnification $\times 250$ (left), and $\times 500$ (right).

(Vaquero et al., 1989, 1990a,b). Although in the present series, only one meningioma showed significant lymphocytic infiltration, the relatively high number of NK-cells in this tumor suggests that a correlation between the degree of lymphocytic infiltration and the presence of IOT-10-positive NK-cells cannot be ruled out in meningiomas.

In any case, the observation of two recurrent meningiomas showing a clear increase in the number of NK-cells, when compared with the original tumors, suggests that an immunological response, related to tumor recurrence and mediated by NK-cells, can occur in meningiomas. On the other hand, the relatively high number of NK-cells found in all the tumor samples of case 17, a female with multiple meningiomas, suggests the possibility of a greater NK-cell-mediated immunological response to these tumors in patients with such entity.

Recently, the presence of macrophages has been reported among tumor cells in meningiomas, and a correlation between presence of macrophages and peritumoral edema has been suggested (Shinanoga et al., 1988). Both macrophages and NK-cells are examples of non-tumoral cells in brain tumors that generally remain elusive because they masquerade among tumor cells, in slices stained by the H & E technique. It is obvious that the identification of these infiltrating non-tumoral cells provides the basis for a better knowledge of the cellular interactions in brain tumors.

In conclusion, our present study shows that, although to a slight and variable degree, IOT-10-positive NK-cells represented a constantly present subpopulation of mononuclear cells in meningiomas. Because our present data suggest that the degree of NK-cell infiltration increases in meningiomas when tumor recurrence occurs, it is necessary to identify both the role of NK-cells in meningiomas, and their possible modulation by different forms of immunotherapy.

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